OIPE
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PATENT & TRADEMARK OFFICE

Marked Up Versions Of Amended Paragraphs:

The paragraph on page 11, lines 3-10:

**Figure 1A**

Amino acid alignment of mouse (SEQ. ID NO:12) and human NADE (HGR74) (4) proteins (SEQ. ID NO:13). The dotted sequence is asparagine rich stretch. The asterisks indicate the leucine-rich nuclear export signal (NES)(5). The closed triangle indicates cysteine residue essential for [dimmer] dimer formation. The prenylation sequence in C-termini is underlined.

The paragraph on page 11, lines 12-19:

**Figure 1B**

Comparison of leucine-rich nuclear export signal (NES) (5) in various protein. The consensus sequence for NES are shadowed. Genbank accession numbers are: cZyxin, X69190 (SEQ. ID NO:14); MAPKK, D13700 (SEQ. ID NO:15); PKI-a, L02615 (SEQ. ID NO:16); TFIIIA, M85211 (SEQ. ID NO:17); RevHIV-1, AF075719 (SEQ. ID NO:18); RanBP1, L25255 (SEQ. ID NO:19); FMRP, L29074 (SEQ. ID NO:20); Gle1, U68475 (SEQ. ID NO:21); RexHTLV-1 ((SEQ. ID NO:22); Human NADE (SEQ. ID NO:23), submitted; mouse NADE (SEQ. ID NO:24), submitted.

The paragraph on page 11, lines 21-22:

**Figure 1C**

Consensus sequence of ubiquitination signal, Mouse (SEQ. ID NO:25); Human (SEQ. ID NO:26) and Consensus (SEQ. ID NO:27).

The paragraph on page 12, lines 10-13:

**Figure 1G-1 and 1G-2**

Blast Search and comparison of mouse NADE nucleic acid sequence

Figure 1G-1 (SEQ. ID NO:28 [__]) and human protein HGR74 sequence (SEQ. ID NO:29).

The paragraph on page 12, lines 15-18:

**Figure 1H**

Comparison of mouse NADE, human HGR74 protein and other homologous rat, mouse and human amino acid sequences: musnade3a (SEQ. ID NO:30); hunade3a1 (SEQ. ID NO:31); hunade3a2 (SEQ.ID NO:32); ratnad3a (SEQ. ID NO:33); ratnad3b (SEQ. ID NO:34); musnade3b (SEQ. ID NO:35); humnade1 (SEQ. ID NO:36); ratnade1 (SEQ.ID NO:37); musnade1 (SEQ. ID NO:38); humnade2 (SEQ. ID NO:39).

The paragraph on page 14, lines 31-35:

**Figure 4A**

At residues 88-100, the mouse NADE NES (SEQ. ID NO:40) lies within the C-terminus. A mouse NADE (SEQ. ID NO:41) is aligned with homologous sequences of NADE family members and the NES sequences of HIV Rev (SEQ. ID NO:42), MAPKK (SEQ. ID NO:43), cZyxin (SEQ. ID. NO:44) and PKI-a (SEQ. ID NO:45).

The paragraph on page 16, line 36 through page 17, line 22:

This invention provides an isolated nucleic molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described isolated nucleic molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor the isolated nucleic acid is a DNA molecule. In another embodiment of the above described isolated nucleic acid molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor the isolated nucleic acid is a cDNA molecule. In a further embodiment of the above described isolated DNA molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor the isolated nucleic acid is a RNA molecule. In an embodiment of the above described isolated nucleic acid molecule

encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, the isolated nucleic acid is operatively linked to a promoter of RNA transcription. In yet another embodiment of the above described nucleic acid molecule, said isolated nucleic acid molecule encodes a neurotrophin associated cell death executor protein. In an embodiment of the above described nucleic acid molecule, said isolated nucleic acid molecule comprises a sequence of AATTG TCTAC GCATC CTTAT GGGGG AGCTG TCTAA C (SEQ.ID NO:1).

The paragraph on page 19, line 17 through page 20, line 11:

This invention provides a vector which comprises the isolated nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, operatively linked to a promoter of RNA transcription. In an embodiment of the invention, where in the vector which comprises the isolated nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, operatively linked to a promoter of RNA transcription is a plasmid. In another embodiment the above described isolated nucleic acid molecule which is a cDNA molecule encoding a polypeptide capable of binding a $p75^{NTR}$ receptor, encodes a human or mouse protein. In yet another embodiment the above described isolated nucleic acid molecule is a cDNA molecule wherein the nucleic acid molecule encodes a polypeptide capable of binding a $p75^{NTR}$ receptor comprising the amino acid sequence set forth in Figure [1G-1]1A (SEQ. ID NO:13 [__]). In a further embodiment the above described isolated nucleic acid molecule is a cDNA molecule wherein the nucleic acid molecule encodes a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described isolated nucleic acid molecule which is a cDNA molecule wherein the nucleic acid molecule encodes a polypeptide capable of binding a $p75^{NTR}$ receptor which is a mouse, rat or human protein. In yet another embodiment of the above described isolated nucleic acid molecule which is a cDNA molecule, said isolated nucleic acid molecule comprises the nucleic acid sequence set forth in Figure

1G-1 (SEQ. ID NO:29 [__]).

The paragraph on page 25, lines 5-32:

This invention provides a purified a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described purified polypeptide capable of binding $p75^{NTR}$ receptor is encoded by the isolated nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment the above described polypeptide capable of binding a $p75^{NTR}$ receptor is a fragment of the purified polypeptide capable of binding a $p75^{NTR}$ receptor. In another embodiment the above described purified polypeptide capable of binding a $p75^{NTR}$ receptor has substantially the same amino acid sequence as set forth in Figure [1G-1] 1A (SEQ. ID NO:13[__]). In a further embodiment the above described purified polypeptide capable of binding a $p75^{NTR}$ receptor having an amino acid sequence as set forth in Figure [1G-1] 1A (SEQ. ID NO:13 [__]). In yet another embodiment the above described polypeptide capable of binding a $p75^{NTR}$ receptor has an amino acid sequence as set forth in Figure [1G-1] 1A (SEQ. ID NO:13 [__]). In a further embodiment, the above described polypeptide capable of binding a $p75^{NTR}$ receptor is a vertebrate polypeptide capable of binding a $p75^{NTR}$ receptor. In an embodiment of the above described polypeptide capable of binding a $p75^{NTR}$ receptor comprises a neurotrophin associated cell death executor protein. In yet another embodiment of the above described polypeptide capable of binding a $p75^{NTR}$ receptor comprises NCLRILMGELSN (SEQ. ID NO:2).

The paragraph on page 26, lines 1-9:

As used herein, a polypeptide capable of binding a $p75^{NTR}$ receptor having "substantially the same" amino acid sequences as set forth in Figure [1G-1] 1A (SEQ. ID NO:13 [__]) is encoded by a nucleic acid encoding a polypeptide capable of binding a $p75^{NTR}$ receptor,

said nucleic acid having 100% identity in the homeodomain regions, that is those regions coding the protein, and said nucleic acid may vary in the nucleotides in the non-coding regions.

The paragraph on page 26, line 29 through page 20, line 1:

This invention provides a polyclonal antibody directed to an epitope of the purified protein having the amino sequence as set forth in Figure [1G-1] 1A (SEQ. ID NO:13 [__]). In a further embodiment the above described monoclonal or polyclonal antibodies are directed to the polypeptide capable of binding a $p75^{NTR}$ receptor, having the amino sequence as set forth in Figure [1G-1] 1A(SEQ. ID NO:13 [__]).

The paragraph on page 59, line 35 through page 60, line 34:

**DNA construction.**

A full length mouse NADE cDNA was constructed on pBluescript II vector by the ligation of the partial NADE cDNA (7-524) and 5'-RACE product. PCR cloning techniques were used to replace the stop codon and add the 5' *Xho*I site and 3' *Bam*HI site of a full length NADE cDNA. pcDNA3.1(-)Myc-HisA/NADE was constructed by insertion of a full length NADE cDNA to *Xho*I-*Bam*HI site of pcDNA3.1(-)Myc-HisA (Invitrogen). Human NADE cDNA was amplified using a Jurkat T cell cDNA library and cloned to pcDNA3.1(-)Myc-HisA pcDNA3/rat $p75^{NTR}$ was constructed by insertion of a full length rat $p75^{NTR}$ cDNA to *Eco*RI site of pcDNA3(Invitrogen). pGEX4T-1/rat $p75^{NTR}$ICD was constructed by insertion of amplified rat $p75^{NTR}$ICD(a. a. 338-396) to pGEX4T-1(Pharmacia). Mutant NADE expression plasmids, pcDNA3.1(-)Myc-HisA/muNADE (Cys102Ser) and pcDNA3.1(-)Myc-HisA/muNADE(Cys121Ser), were constructed by PCR-based site-direct mutagenesis methods (29).

pELAM-Lu for luciferase reporter assay was constructed by insertion of NF-κB binding site of E-selectin promoter region (-730 - 52) to pGL3-Basic *Sac*I-*Bgl*II site. Expression plasmids of GFP-

fused NADE proteins were made following: The cDNA of GFP was cloned into NheI-XhoI-cut pcDNA3.1-mouse NADE as a PCR product amplified with the primers 5"-CTAGCTAGCATCATGGTGAGCAAGGGCGAG-3" (SEQ. ID NO:3) and 5"-CCGCTCGAGTCTTGTACAGCTCGTCCAT-3" (SEQ. ID NO:4) using pEGFP-N2 (Clontech) as a template. The deletion mutants delta 101-124-GFP and delta 91-124-GFP were constructed by inserting an XhoI-BamHI-cut PCR fragment generated with Expand high fidelity Taq polimerase (Boehringer Mannheim) into XhoI-BamHI-cut pcDNA3.1-GFP using the primers
5"-ATCCTCGAGCGATCATGGCCAATGTCCAC-3" (sense)(SEQ. ID NO:5),
5"-ATCGGATCCTCTCAGCTGTAGCTCCCT-3" (antisense) (SEQ. ID NO:6) and
5"-ATCGGATCCGATCTCTCTCATCTCCTC-3" (antisense)(SEQ. ID NO:7).

The paragraph on page 60, line 36 through page 61, line 6:

The mutagenic primers
(5'-AAAGCTTAGGGAGGCACAGCTGAGAAA-3" (SEQ. ID NO:8),
5"-TTTCTCAGCTGTGCCTCCCTAAGCTTT-3" (SEQ. ID NO:9),
5"-ATCCGGAGAAAGGCTAGGGAGGCACA-3" (SEQ. ID NO:10),
and 5"-TGTGCCTCCCTAGCCTTTCTCCGGAT-3")(SEQ. ID NO:11)
were used to obtain L97A-GFP and L94, 97A-GFP in which Leu94 and Leu97 are replaced with Ala. In all constructs, mutations were verified by sequencing.